Inhibition of D-Ala Incorporation During Wall Teichoic Acid Biosynthesis by Desleucyl-Oritavancin in Staphylococcus aureus

J. Chang, a L. Coffman, a and S. J. Kim a

 a Department of Chemistry, Baylor University, One Bear Place #97348, Waco, Texas 76798 United States.

 a E-mail: Sung_J_Kim@baylor.edu.

Experimental methods

Growth of S. aureus and incorporation of 13C and 15N-isotope labels. Detailed growth condition and the composition for S. aureus Standard Medium (SASM) are described elsewhere. 1,2 Briefly SASM contained the following per litre: 10 g of d-glucose; 1 g each of K2HPO4·3H2O, KH2PO4, and (NH4)2SO4; 0.2 g of MgSO4·7H2O; 10 mg each of MnSO4·H2O, FeSO4·H2O, and NaCl; 5 mg each of adenine, cytosine, guanine, uracil, and xanthine; 2 mg each of calcium pantothenate, thiamin hydrochloride, and niacin; 1 mg each of pyridoxine hydrochloride, riboflavin, inositol, CuSO4·5H2O, and ZnSO4·7H2O; 0.1 mg each of biotin and folic acid; and 0.1 g of all 20 common amino acids. All amino acids and chemicals used for SASM were purchased from Sigma-Aldrich (St. Louis, MO). Overnight culture of S. aureus (strain ATCC 6538P) was added to 1% final volume to an one-liter flask containing 300 mL of SASM. The natural abundance amino acids L-Ala and L-Lys were replaced with L-[1-15N]Ala or D-[1-15N]Ala, and L-[1-13C]Lys. The stable isotope labelled amino acids were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). PG bridge-link (Fig. 1a) was examined through 13C-15N spin pair labelled by growing S. aureus in SASM containing L-[1-15N]Ala and L-[1-13C]Lys. PG and WTA in S. aureus were differentially 15N labelled by growing S. aureus in SASM containing D-[1-15N]Ala and L-Ala with alanine racemase inhibitor alaphosphin (Sigma-Aldrich) to final concentration of 5 µg/mL to prevent the racemic conversion between L-Ala and D-Ala. Cells were grown in 37 °C with 250 rpm shaking in a Environ-Shaker (Lab-Lines Instruments, Inc., Melrose Park, IL), with des-Ori (20 µg/mL) added at the mid-exponential growth phase. Cells were harvested after 1 hr of growth by centrifugation at 8,000g for 10 min at 4°C in a Sorvall GS-3 rotor. Harvested cells were washed twice with deionized water and lyophilized.

Solid-state NMR spectrometer and REDOR analysis. REDOR NMR experiments were performed on intact S. aureus at 7.0 T (300 MHz for 1H, 75 MHz for 13C, and 30 MHz for 15N) provided by 89-mm bore Oxford (Cambridge, U.K.) superconducting solenoids. The four-frequency transmission-line probe was equipped with Chemagnetics/Varian magic-angle spinning ceramic stator with a 14-mm long, 9-mm inner-diameter sample coil. The samples were spun in room temperature at 5 kHz (maintained within ±2 Hz). Radio-frequency π-pulse lengths were 10 µs for both 13C and 15N. Proton-carbon and proton-nitrogen matched cross-polarization transfers were at 50 kHz for 2 ms. Proton dipolar decoupling during the signal acquisition was 105 kHz. The re-cycle delay period was 2 seconds. Detailed experimental setup is described elsewhere. 2

REDOR is a solid-state NMR method that directly measures dipolar couplings between two nuclei, which allows inter-nuclear distances between isolated spins to be determined 3. REDOR is a difference experiment between two collected spectra, one in the absence of heteronuclear dipolar coupling (full echo, S0 spectrum), and other in the presence of coupling (dephased echo, S spectrum). The difference in signal intensity (ΔS = S0 - S) is directly related to the heteronuclear dipolar coupling. REDOR analysis used modified Bessel function expressions for an IS spin-1/2 pair 4.

ATP leakage assay. ATP leakage assay was performed on overnight cultures of S. aureus (ATCC 6538P) grown in tryptic soy broth harvested at OD600nm of 1.5. Cells were first pelleted by centrifugation at 7200g for 5 min, then resuspended in pH 7.4 phosphate buffered saline with the glycopeptide antibiotic
added to final drug concentrations of 0, 0.5, 1, 2, 5, 10, 50, and 100 µg/mL. Cells were incubated with the antibiotic for 20 min at 37 °C. After the incubation, bacteria were pelleted by centrifugation at 7200g for 5 min and the supernatant was analysed for the ATP. The ATP leakage amount was measured by adding 100 µL of CellTiter-Glo® 2.0 reagent (Promega, Madison, WI) to equal volume of the supernatant. After 10 min of equilibration, luminescence from the mixture was measured using Fluoroskan Ascent FL Luminometer (Thermo Scientific) with the integration time of 200 ms.

References